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COMPLETE SPECIFICATION

Insulin Preparations and Process for Preparing same

We, N.V. ORGANON, a body corporate under the laws of Holland, of 6, Kloosterstraat, Oss, Holland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

This invention relates to new insulin-containing preparations, the effect of which on the sugar metabolism is more favourable than that of the known insulin preparations. More particularly, the invention concerns injectable metal-containing preparations of insulin and glucagon.

It is known that in a healthy body the hormone insulin plays an important part in the sugar metabolism and in connection therewith in the regulation of the blood sugar content. If this hormone is not present in a sufficient measure, disturbances will occur in the sugar metabolism and in processes connected therewith. The result may be, among other things, that the blood sugar content rises above the normal value with all undesired consequences thereof, such as the secretion of glucose in the urine. By administration of insulin, which will usually take place subcutaneously or intramuscularly, the normal sugar metabolism can be restored somewhat in patients and in this manner too high a blood sugar content can be reduced; moreover, measures can be taken to prevent the blood sugar content from rising too strongly above its normal value.

Further, it is known that in the body still other substances occur which exert an influence on the blood sugar content. One of these is glucagon, a hyperglycemic-glycogenolytic factor of which it is known that it is capable of raising the blood sugar content by promoting the conversion of glycogen stored in the liver into glucose. It may, for example, be administered to patients with hyperinsulinism and to patients that are in an insulin coma, for example in the treatment of schizophrenics who undergo insulin shock therapy. In nearly all the cases the coma will be ended by this administration. In connection herewith the glucagon is in general considered an antagonist of the insulin. As a result thereof, in the preparation of insulin efforts have been made to achieve removal of the glucagon as completely as possible.

However, the action of the glucagon is not only a blood sugar increasing action. It promotes at the same time, in collaboration with the insulin, the consumption of glucose by the muscles, as a result of which the latter are better enabled to make use of it for all kinds of processes. In the normal organism there is present in this manner a combination of the effects of the glucagon and the insulin. On the one hand, the glucagon causes the liberation of glucose from the liver glycogen, as a result of which glucose is made available, while on the other hand glucagon stimulates the consumption of glucose. As one of the effects of the insulin consists in the conversion of glucose into glycogen, the blood sugar content is kept between certain values partly due to the equilibrium between these different effects.

If by some deviation or other a decreased sugar consumption should arise, this may partly be restored by administering insulin. Usually the quantity of insulin to be administered is ascertained as accurately as possible in each patient, while the same is done to the diet. Since, however, administration of insulin only results in a one-sided influencing of the sugar metabolism, the latter will, in diabetics, often be subject to great fluctuations, in spite of the supply of insulin. As a result, they will in general not have the disposal of a sufficient quantity of glucose, whenever they are required to perform extraordinary activities.

By administering insulin in combination with glucagon it is possible to place a larger quantity of glucose at the disposal of the

organism, without the blood sugar content rising to such an extent that a great part of the glucose is secreted again.

Previously this effect could only be attained by administering a mixture of insulin and glucagon by means of a prolonged intravenous infusion.

In accordance with the present invention, a process has been found for the preparation of new, insulin-containing injectable preparations, which contain glucagon in addition to insulin, which process is characterized in that an injectable preparation is prepared containing insulin, glucagon, and at least one of the metals zinc, cobalt, nickel, copper, manganese, aluminium, and iron, the glucagon being present in a quantity at least 1% of the weight of the insulin, while the metal is present in a quantity of at least 0·3 milligram equivalent per gram of the combination of insulin and glucagon, the *p*H of the preparation being adjusted to a value between about 2 and 9. The combination of insulin, glucagon and the metal is distributed in an aqueous injectable medium.

Salts useful for the preparations of the present invention are the salts of the mentioned metals with pharmaceutically acceptable anions such as acetates, propionates, sulphates, chlorides and bromides. Of the mentioned salts the acetates are to be preferred.

By an injectable medium is understood a sterile solution which may contain pharmaceutically-acceptable substances which make the solution substantially isotonic, such as sodium chloride and glycerol as well as a preservative such as phenol or tricresol, while the *p*H of the solution is not such that on injection serious damages of the tissue occur.

By the weight of insulin and glucagon is understood the weight which the quantities present would have if they were present in a pure form.

The mixture of insulin and glucagon present in the thus prepared preparations has the property of being insoluble in aqueous medium at physiological *p*H. That this would be the case could not be foreseen, because a mixture of glucagon and zinc without insulin is only partly insoluble under the same conditions. As a result, in the present preparations not only the insulin but also the glucagon has a prolonged activity. The mixture of glucagon and insulin is hereinafter indicated by the term *combination*, it being understood that this term is not intended to indicate that a chemical interaction between the insulin and the glucagon has taken place. It is, however, not improbable that in the precipitates of the combination of insulin and glucagon the molecules of the two substances are linked together by means of metal atoms. The crystalline precipitates which are described hereinafter most probably consist of precipitates of metal containing insulin in which a certain amount of metal containing glucagon has been included. The same holds good for the amorphous precipitates in which the glucagon probably is included in the amorphous particles of the insulin.

A more effective use of carbohydrates is obtained when applying the preparations of the present invention than when applying preparations containing insulin only. As a result the patient is enabled to accomplish greater activities than is possible on treatment with the thus far commonly used preparations.

The *p*H of the present preparations may be between about 2 and 9. Preferably a *p*H will be chosen which does not deviate too much from the *p*H of the tissue fluids. It has been found that the *p*H range between 6 and 8 is very advantageous. In this range the active substances are present in a suspended form. The type of action of these suspensions may be varied by giving the suspended particles a certain form. The action, for example, of a suspension in which the precipitate is amorphous will be less prolonged than that of a suspension in which it is crystalline. The type of action may also be varied by providing for the presence of a precipitate which is partly amorphous, and partly crystalline. Furthermore, the same result can be obtained by selecting suspended particles having a certain size range, which can be effected by applying special crystallization techniques or by making use of mechanical methods, such as the grinding of a coarse precipitate. The particle-size of the crystals may vary from about 0·1 to about 100 μ, the prolonged action of the very small particles being less pronounced than those of the large crystals. The very large crystals (of sizes exceeding 100 μ) have the disadvantage of rapid settling of the precipitate. For practical purposes sizes of from 10 to 40 μ are to be preferred.

On adjusting the *p*H to a value in the range of from about 6–8, use will frequently be made of a buffering substance, because of this means the occurrence of undesired *p*H shifts is prevented, as a result of which the type of action of the preparation might be changed.

The ratio by weight of insulin: glucagon in the present preparations is preferably chosen between about 0·5 : 1 and 20 : 1. If this ratio exceeds 100, little indication is any longer observed of a favorable effect on the carbohydrate metabolism compared with the usual insulin preparations.

If the medium does not contain any compounds that have a greater affinity for the said metals than insulin and glucagon, a quantity of metal should be present of at least 0·3 milligram equivalent per gram of the combination of insulin and glucagon. If such metal binding compounds are present indeed,

the minimum of metal will have to be chosen higher. Preferably the metal content will be chosen in the range of from about 1 to 10 milligram equivalent per gram of the combination of insulin and glucagon.

In some cases it is desirable to add to the preparations with a *p*H in the range of from about 6–8 a complex former for the metal present. It is desirable not to select such a strong complex former or such quantities thereof that all the metal is bound by it so that no metal is any longer available for the insulin and glucagon. As examples of useful complex formers are mentioned glycine, leucine, aspartic acid, glutamic acid, malic acid and tartaric acid. By the presence of the complex former in the present suspensions the result is brought about that a latent supply of metal is present, as a result of which under certain conditions metal ions can be supplied, which consequently results in the metal concentration being buffered. As a result of this mixing with other insulin preparations, especially with clear insulin solutions which are poor in zinc, is readily possible to vary the type of action. Moreover, in the preparations in which an α-amino acid, for example glycine, is applied, no undesired *p*H shifts can occur, owing to the buffering effect of the soluble complex of the metal with the α-amino acid.

Addition to the present preparations of compounds delaying the resorption is possible. As a result it is possible to prolong the effect of the preparations still further. Compounds useful for this purpose are, for example, gelatin and dextrans, which substances are known to delay the resorption of injected medicaments.

Preferably substances of the type that are commonly used in injection preparations are added to the preparations, such as a preservative, for example phenol, Nipagin (registered Trade Mark) (methyl p-hydroxybenzoate), and tricresol, and an isotonisation agent, such as sodium chloride, sodium acetate and glycerol.

Of the metals mentioned previously zinc is preferred.

There are many possibilities of variation in the sequence in which the various components of the present preparations are added to each other. This holds good both for the preparation of solutions and of suspensions of amorphous, or crystalline particles. It is, for example, possible to dissolve the desired quantities of glucagon and metal in an acid insulin solution. If desired, the *p*H can then be brought at a value of, for example, 7. If the *p*H is adjusted to this value very rapidly, the precipitate will be formed in an amorphous form; if this treatment is carried out more slowly or stepwise, the precipitate will be formed entirely or partly in a crystalline form.

Another mode of preparing the present preparations in the form of crystal suspensions is that in which insulin, glucagon, and a metal salt all in acid solution are added to each other, subsequently the acidity is adjusted to a value of about 6, and the mixture is left to stand at this value until crystallisation has taken place in a sufficient measure, after which, if desired, the *p*H is raised to the physiological value.

It is self-evident that on preparing the preparations according to the invention the necessary measures must be observed by which sterility of the final products is assured.

The following examples illustrate the invention. Needless to say, the invention is not to be restricted to these examples, it being possible to apply many variations thereon without departing from the scope of the invention.

EXAMPLE 1

156·5 mg of insulin powder (biological activity 25·6 U/mg, zinc content 0·42%) and 80 mg of pure and crystalline glucagon are dissolved in 40 ml 0·01 N hydrochloric acid containing 0·1% Nipagin (Registered Trade Mark). By means of 0·1 N sodium hydroxide solution this solution is adjusted to *p*H 3·0.

To this solution are added 40 ml of an aqueous solution containing 2650 mg of glycerol, 32·5 mg of zinc acetate 2 aq. and 60 mg of Nipagin (Registered Trade Mark) which solution is adjusted to *p*H 3·0 with 0·1 N hydrochloric acid. Then, by means of distilled water which has been adjusted to *p*H 3·0 with 0·1 N hydrochloric acid the volume is completed to 100 ml.

EXAMPLE 2

326·5 mg of zinc-free insulin powder (biological activity 24·5 U/mg) and 40 mg of pure and crystalline glucagon are suspended in 10 ml of distilled water. While stirring, sufficient 1 N hydrochloric acid is added dropwise so that the protein goes into solution entirely. Then the volume is brought to 20 ml by means of distilled water. This solution is dispensed in quantities of 2 ml in suitable vials and lyophilized.

Then, per vial of this lyophilized mixture of insulin and glucagon, 10 ml of solvent are added having the following composition: —

Glycerol	2550 mg
Cobaltous chloride 6 aq	58·2 mg
Tricresol	300 mg
Hydrochloric acid 0·1 N to *p*H 2·0	—
Distilled water to	100 ml

After shaking, a solution is obtained suitable for injection.

EXAMPLE 3

In 40 ml of distilled water, containing 0·1% Nipagin (Registered Trade Mark) are suspended 334·8 mg of crystalline insulin (biological activity 23·9 U/mg, zinc content 0·52%) and 400 mg of 80% pure glucagon. Subsequently, while stirring, so much 1 N

hydrochloric acid is added as is necessary to dissolve both substances completely. Then 40 ml of an aqueous solution are added in which there are present 850 mg of glacial acetic acid, 63 mg of zinc chloride 0 aq., and 60 mg of Nipagin (Registered Trade Mark).

While stirring vigorously the *p*H is rapidly adjusted to a value of 7·1 by means of 1 N sodium hydroxide solution. The volume of the suspension, in which only amorphous particles are present, is finally completed to 100 ml with distilled water. The precipitate consists of a mixture of zinc containing insulin and zinc containing glucagon.

EXAMPLE 4

In the manner of Example 3 a preparation is prepared containing 99 mg of manganese dichloride 2 aq. instead of 63 mg of zinc chloride 0 aq.

EXAMPLE 5

In the manner of Example 3 a preparation is prepared containing 145 mg of nickel chloride 6 aq. instead of 63 mg of zinc chloride 0 aq.

EXAMPLE 6

In 5 ml of an aqueous solution containing 400 mg of glacial acetic acid there are dissolved successively 156·3 mg of crystalline insulin (biological activity 25·6 U/mg, zinc content 0·42%) and 40 mg of pure, crystalline glucagon. Then 50 ml of an aqueous solution are added in which are present 40 mg of glacial acetic acid, 150 mg of glycine, 92 mg of zinc acetate 2 aq., and 60 mg of Nipagin (Registered Trade Mark).

While stirring regularly, 0·5 N sodium hydroxide solution is added within a few minutes until the *p*H amounts to 5·9.

Then the liquid is allowed to stand at room temperature for four days, while stirring twice daily, in which period crystals are formed.

Then, while stirring vigorously, 0·5 N sodium hydroxide solution is carefully added until the *p*H has reached a value of 7·2. Then 1400 mg of glycerol and 40 mg of Nipagin (Registered Trade Mark) dissolved in distilled water to 20 ml, are added and the volume is brought to 100 ml with distilled water.

The thus obtained suspension predominantly consists of crystalline particles.

EXAMPLE 7

156·2 mg of the crystalline insulin mentioned in Example 6 and 30 mg of crystalline glucagon are dissolved in 5 ml of an aqueous solution containing 400 mg of glacial acetic acid. Subsequently 50ml of an aqueous solution are added in which 40 mg of glacial acetic acid, 150 mg of glycine, 92 mg of zinc acetate 2 aq., and 60 mg of Nipagin (Registered Trade Mark) have been dissolved.

While stirring slowly and regularly, 0·5 N sodium hydroxide solution is added in such a manner that the *p*H rises from about 3 to a value of 7·3 in about 24 hours. Added are then 1400 mg of glycerol and 40 mg of Nipagin (Registered Trade Mark) dissolved in 20 ml of distilled water, and the final volume is brought to 100 ml with distilled water.

As in Example 6, an approximately isotonic suspension is formed with predominantly crystalline particles.

EXAMPLE 8

194·2 mg of the precipitate obtained according to the method mentioned in Example 7 and containing 15% of glucagon, are suspended in an aqueous solution of *p*H 7·0, consisting of: —

Zinc acetate 2 aq.	65 mg
Glycerol	2800 mg
Phenol	500 mg
Distilled water to	100 ml

The resulting suspension has an activity which is comparable to that of the crystal suspensions described in Examples 6 and 7 and contains 40 U of insulin per ml.

EXAMPLE 9

To a solution of 165 mg of crystalline insulin (biological activity 24·2 U/mg, zinc content 0·47%) and 80 mg of crystalline glucagon in 5 ml of 0·1 N hydrochloric acid, there are added 85 ml of a solution containing 300 mg of tricresol, 760 mg of sodium chloride, 150 mg of glycine and 45 mg of aluminium (in the form of aluminium chloride).

By means of 1 N sodium hydroxide solution the *p*H is rapidly adjusted to 7·5. The volume of the formed suspension of amorphous particles is completed to 100 ml with distilled water.

EXAMPLE 10

To a solution of 156·2 mg of crystalline insulin (biological activity 25·6 U/mg, zinc content 0·42%) and 5 mg of pure, crystalline glucagon in 5 ml of 0·1 N hydrochloric acid there are added 85 ml of a solution containing 500 mg of phenol, 760 mg of sodium chloride and 4·2 mg of zinc chloride 0 aq. By means of 1 N sodium hydroxide solution the *p*H of the solution is then rapidly adjusted to a value of 7·1, after which the volume is brought to 100 ml with distilled water.

The resulting suspension contains amorphous particles.

WHAT WE CLAIM IS: —

1. An injectable insulin containing preparation comprising insulin, glucagon, and at least one metal selected from the group consisting of zinc, cobalt, nickel, copper, manganese, aluminium, and iron, distributed in an aqueous injectable medium, the glucagon being present in said preparation in a quantity of at least 1%, of the weight of the insulin, and the metal being present in a quantity of at least 0·3 milligram equivalent per gram of insulin and glucagon, the *p*H of said preparation being between about 2 and 9.

2. An injectable insulin containing preparation comprising insulin, glucagon, and at least one metal selected from the group consisting of zinc, cobalt, nickel, copper, manganese, aluminium, and iron, distributed in an aqueous injectable medium, the ratio by weight of insulin to glucagon being between about 0·5 : 1 and 20 : 1, and the metal being present in a quantity ranging between about 1 to 10 milligram equivalents per gram of insulin and glucagon, the *p*H of said preparation being between about 2 and 9.

3. An injectable insulin containing preparation comprising an insoluble combination of insulin, glucagon, and at least one metal selected from the group consisting of zinc, cobalt, nickel, copper, manganese, aluminium and iron, in suspension in an aqueous injectable medium, the glucagon being present in said preparation in a quantity of at least 1% of the weight of the insulin, and the metal being present in a quantity of at least 0·3 milligram equivalent per gram of insulin and glucagon, the *p*H of said preparation being between about 6 and 8.

4. The preparation of Claim 1 in which the metal is zinc.

5. The preparation of Claim 1 in which there is present a complex former for the metal, comprising an α-amino acid.

6. The preparation of Claim 5 in which the amino acid is glycine.

7. The method of producing an injectable insulin containing preparation which comprises admixing insulin, glucagon, and an acid solution of a salt of a metal selected from the group consisting of zinc, cobalt, nickel, copper, manganese, aluminium, and iron, and adjusting the *p*H of the preparation to a value between about 2 and 9, the glucagon being present in a quantity of at least 1% of the weight of the insulin, and the metal being present in a quantity of at least 0·3 milligram equivalent per gram of insulin and glucagon.

8. The method of Claim 7 in which the metal is zinc.

9. The method of Claim 7 in which an α-amino acid is added as a complex former for the metal.

10. The method of Claim 7 in which the *p*H value of the preparation is adjusted to between about 6 and 8 to permit separation of an insoluble combination of insulin, glucagon and metal.

11. Methods of producing insulin containing preparations, substantially as herein described with reference to the foregoing examples.

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